Theoretical Section – What Every Student Should Know:

1. The most common etiological agents of sexually transmitted infections/diseases - systematized according to groups of microorganisms (bacteria, viruses, fungi, parasites), bacterial groups (Gram-negative, Gram-positive, atypical, anaerobic, spirochetes), and frequency of occurrence.
2. Clinical course of STDs - the most common clinical presentations of infections (with emphasis on the relationship between presentation and appropriate sampling for diagnostic testing); differences in the course of STDs in women and men; high-risk groups; the most frequent complications of STDs.
3. Vertical transmission of STDs - congenital and perinatal infections.
4. Treatment of STDs - major antibiotic groups and drugs of choice, including antifungal and antiviral agents.
5. Epidemiology of STDs - reservoirs and sources of infection, routes of transmission classified as: exclusively sexual contact, sexual and close physical contact; other routes with examples of diseases and their causative pathogens.
6. Diagnostics of STDs - in which infections microscopy (type of microscopy) is of value; in which infections culture is a routine method and when it is optional; likewise, when molecular tests are the primary diagnostic tool and when they serve as an adjunct; when serology is useful in STD diagnosis; and when diagnosis has limited importance due to pathognomonic clinical features. From which anatomical sites should microbiological samples be collected? In which STD presentations should blood be collected? Can cerebrospinal fluid serve as diagnostic material in STDs? Under what conditions should diagnostic samples be transported?
7. Specific and non-specific prevention of STDs (general overview).

DIAGNOSIS OF SEXUALLY TRANSMITTED INFECTIONS (STIs)

The types of clinical material collected for diagnosing venereal diseases are highly diverse. Methods of sample collection depend primarily on the anatomical location of the lesions. The table below summarizes the disease entity, the method of specimen collection, and the diagnostic techniques applied.

Syphilis	
Clinical specimens	**Sample collection**
Material from ulcers, papular or maculopapular rash	Active or moist lesions should be carefully cleansed with a sterile swab moistened with sterile saline. After gently drying the lesion with a sterile swab, apply gentle pressure to obtain a serous exudate. The collected exudate should be placed on a microscope slide using a stainless steel/platinum spatula or a disposable loop and examined with dark-field microscopy, or allowed to air-dry and sent to the laboratory (secured in a sterile container). For molecular testing, the exudate should be transferred into a dedicated transport medium or frozen if immediate testing is not possible.
Blood serum	Whole blood is collected into a sterile, plain tube (“clot tube”). After clot formation, serum is separated using a pipette (or syringe). The obtained serum should be stored at refrigerator temperature until sent to the laboratory.
Cerebrospinal fluid	In suspected neurosyphilis, cerebrospinal fluid is obtained by lumbar puncture for diagnostic testing.
Tissue biopsies from skin lesions, mucous membranes, or placenta.	Secure in a tightly sealed sterile container with sterile saline to prevent drying.
	Diagnostic tests
	Microscopy – observation of live spirochetes by dark-field microscopy (not routinely used); fluorescence microscopy: immunofluorescence on fixed smears from inflammatory exudate. Serology a) cardiolipin tests (VDRL, USR, RPR) – detection of reagin (non-specific antibodies directed against cellular lipoproteins) b) treponemal tests (FTA-ABS, passive hemagglutination) Molecular tests (NAAT) – from skin or mucosal lesions, tissue biopsies. Immunohistochemistry – from skin lesions, hard chancre, placenta, and mucosal lesions.

Gonorrhoea	
Clinical specimens	**Sample collection**
Cervical discharge/cervical swab	The specimen is collected using a vaginal speculum. Before sampling, the external opening of the cervical canal should be carefully cleaned with a sterile swab. For testing, a pathological discharge or a swab from the cervical wall is obtained. The swab is inserted 2–3 cm into the cervical canal and gently rotated a full 360° for 5–10 seconds. IMPORTANT: Cervical canal samples should not be collected from prepubertal girls or from women after hysterectomy (surgical removal of the uterus); in such cases, material should be obtained from the vaginal vestibule, or a urine specimen should be used.
Urethral discharge	Urethral samples should be collected at least one hour after the last urination. In women, the external urethral orifice should be cleaned with a swab moistened with sterile saline. In men, the foreskin should be retracted to avoid contamination with skin microflora during sampling. Discharge is collected directly onto a swab. If no exudate is present, a thin swab is inserted 2-3 cm into the urethra and gently rotated for 5-10 seconds.
Pharyngeal swab	Swabs are collected from the posterior pharyngeal wall, above the lower margin of the soft palate, from both tonsils and tonsillar crypts, as well as from any lesions.
First-void urine	Uncentrifuged first-void urine, collected without prior cleansing of the genital area, was placed in a sterile container for molecular testing.
Penile swab	Collect only if other specimens (e.g., urine) cannot be obtained.
Rectal swab	Insert the swab 2-3 cm into the rectum and rotate, touching all rectal walls for 10 seconds (take care to avoid fecal contamination).
Conjunctival swab	Retract the lower eyelid and swab the surface of the lower palpebral conjunctiva toward the medial canthus.
	Diagnostic tests
	Microscopy – smear stained with the Loeffler or Gram method; observation of diplococci inside leukocytes. Culture - inoculation of discharge samples transported in special transport media (sensitive to drying) and plated on selective media for gonococci. Molecular tests (NAAT).
Chlamydiosis (Chlamydia infection)	
Clinical specimens	**Sample collection**
First-void urine	Uncentrifuged first-void urine, collected without prior cleansing of the genital area, was placed in a sterile container for molecular testing. For immunofluorescence – sediment from centrifuged urine.
Urethral /cervical swab with collection of epithelial cells	Urethral samples should be collected at least one hour after the last urination. For testing, a swab is taken from inside the urethra. A thin, flexible swab is inserted 2-3 cm into the urethra and gently rotated 360° for 5-10 seconds to obtain epithelial cells. • Due to the frequent co-infection with Chlamydia trachomatis and gonorrhoea, the order of sampling is essential: discharge should first be collected for gonorrhoea testing, followed by epithelial scrapings (urethral swab) for chlamydia testing.
Blood serum	Serology should be used only as an adjunct in the diagnosis and screening of complicated Chlamydia trachomatis infections (reactive arthritis, pelvic inflammatory disease, ectopic pregnancy, tubal infertility), neonatal pneumonia, and lymphogranuloma venereum (LGV). Serology should not be used in the diagnosis of uncomplicated urogenital C. trachomatis infection.
	Diagnostic tests
	Molecular tests Detection of Chlamydia antigens: ELISA, direct immunofluorescence, and rapid immunochromatographic assays.
Genital mycoplasmas	
Clinical specimens	**Sample collection**
Urethral swab, endocervical swab (with collection of epithelial cells).	As described above.
	Diagnostic tests
	Culture and Biochemical Methods Molecular Testing

Genital Herpes	
Clinical specimens	**Sample collection**
Fluid from vesicles/exudate from ulcer.	
Blood serum	Serum samples for serological testing (specific antibody detection) should be collected both at the onset of infection (acute phase) and during convalescence (after 2–4 weeks of illness).
CSF	Lumbar puncture
Scrapings	Smear from scrapings collected with a sterile scalpel from the base of erosions or vesicular lesions for the Tzanck test.
Swab from anogenital or oral lesions.	
Swab from the conjunctiva or cornea.	
	Diagnostic tests
	Molecular tests (material from genital ulcers, skin and mucosal lesions, cerebrospinal fluid, and blood in disseminated infections) Serology – EIA, indirect immunofluorescence, Western blot Microscopy (Tzanck test or direct immunofluorescence in herpetic keratitis)
Genital warts (HPV)	
Clinical specimens	**Sample collection**
Genital swab	Collected from the genital tract, urethra, or pharynx using a swab moistened with physiological saline.
Throat/oral swab	Swabs should be collected from the posterior pharyngeal wall, above the lower margin of the soft palate, and from tonsillar crypts, as well as from lesions when present (or from the oral cavity in cases of condylomata acuminata).
	Diagnostic tests: molecular tests
Other genital viruses	
	Sample collection
	Pathogenic viruses may be detected in various blood components, including cells, plasma, and serum (e.g., HIV in lymphocytes and macrophages, CMV in neutrophils, enteroviruses in plasma and white blood cells). For molecular detection of viruses, blood should be collected into Vacutainer tubes containing an anticoagulant (heparin or citrate).
	Diagnostic tests: molecular tests
Bacterial vaginosis (BV) is not classified as a sexually transmitted disease (STD). It is an endogenous infection.	
Clinical specimens	**Sample collection**
Vaginal discharge	The swab should be collected from the irritated mucosa. The swab should be rotated toward the posterior vaginal fornix for approximately 5 seconds.
	Diagnostic tests
	Microscopic examination of vaginal discharge Measurement of vaginal pH Amine (whiff) test.

TASKS to be completed during laboratory classes

I. CASE ANALYSIS

Case 1.

A 25-year-old patient presented to a primary care physician with the following clinical symptoms: profuse purulent urethral discharge accompanied by burning sensation and pain during urination. In the medical history, the patient reported having had sexual intercourse with a newly acquainted casual partner within the past two weeks. On examination, the patient was diagnosed with epididymitis and anterior urethritis.

Questions for students:

1. What do the medical history and physical examination findings suggest?

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2. Which confirmatory tests for the preliminary diagnosis can be performed by a gynecologist?

3. What type of specimen should be collected for microbiological testing—specify the method of collection and the conditions required for its transport to the laboratory. Is a blood culture reasonable in this case?

4. Propose therapeutic options for the case described.

Case 2.

A 24-year-old woman presented to a gynecology clinic. Over the past 72 hours, she developed several painful vesicles within the vagina and in the surrounding area. She reported dysuria, difficulty walking due to inflamed and ulcerating vesicular lesions, fever (38 °C), and general malaise. On examination, bilateral tender inguinal lymphadenopathy and cervical ulcers were observed.

Questions for students:

1. Can a urogenital tract infection be suspected in this patient? If so, which specific infections should be considered? Justify your answer.

2. How can the suspected infection in this patient be confirmed?

3. Should this infection be treated? Explain your answer.

Case 3.

An outpatient, 40 years of age, presented to a gynecologist with complaints of vaginal discharge and dysuria. She also reported itching and burning in the periurethral area. Medical history revealed a recent course of amoxicillin–clavulanate therapy for bronchitis, as well as the use of oral hormonal contraception. On gynecological examination, marked inflammation was observed, with erythema of the vulva and periurethral region, accompanied by profuse, white, curd-like vaginal discharge.

Questions for students:

1. What do the medical history and physical examination findings suggest? Justify your answer.

2. What type of specimen should be collected for microbiological testing (is a blood culture reasonable in this case)? Describe the method of collection and conditions of transport.

3. Should this infection be treated? Explain your reasoning.

II. INTERPRETATION OF VAGINAL BIOCENOSIS

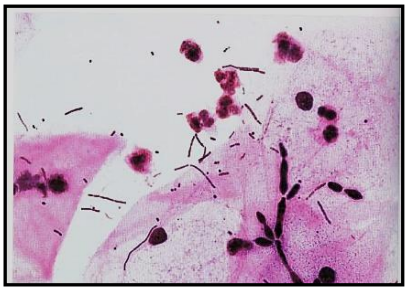
Example of vaginal microbiota assessment (vaginal biocenosis) – Gram-stained vaginal smear from a healthy woman. Magnification 400×. Normal epithelial cells are visible, along with Gram-positive elongated rods of Lactobacillus spp. and a few Gram-positive streptococci. In general, apart from Lactobacillus rods, other bacteria are scarce or absent. The microscopic image also shows no inflammatory cells or fungal elements. The morphology of epithelial cells is normal.

The assessment of vaginal microbiota allows the detection of inflammation and infection.

Evaluation Criteria for Gram-Stained Vaginal Smears:

Leukocytes: absent, single (≤3 per high-power field, HPF), few (4–10 per HPF), increased (>10 per HPF)
Lactobacillus spp.: absent, single (≤10 per HPF), few (11–50 per HPF), numerous (>50 per HPF)
Other bacteria: absent, single (≤10 per HPF), few (11–50 per HPF), numerous (>50 per HPF)
Fungi: absent, single (≤3 per HPF), few (4–10 per HPF), increased (>10 per HPF)
Epithelial cells: normal, clue cells

Case EXAMPLE. Assess the vaginal microbiota from a smear with a known pH in the documented microscopic cases below (enter: increased number, absent, normal, etc.).



Leukocytes – increased number (>10 per field)
Lactobacillus spp. – few
Other bacteria – a few Gram-positive cocci
Yeast cells – increased number (>10 per field)
Epithelial cells – normal
pH – 5.5

Interpretation:

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Case 1. Assess the vaginal microbiota from a smear with a known pH in the following documented microscopic cases (enter: increased number, absent, normal, etc.).

Leukocytes – .....
Lactobacillus spp. – .....
Other bacteria – .....
Yeast cells – .....
Epithelial cells – .....
pH – .....

Interpretation:

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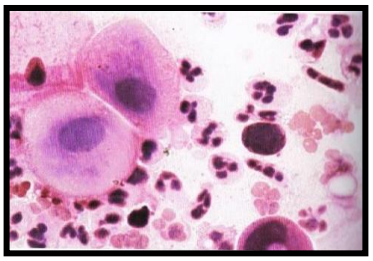
Case 2. Assess the vaginal microbiota from a smear with a known pH in the following documented microscopic cases (enter: increased number, absent, normal, etc.).



Leukocytes – .....
Lactobacillus spp. – .....
Other bacteria – .....
Yeast cells – .....
Epithelial cells – .....
pH – .....

Interpretation:

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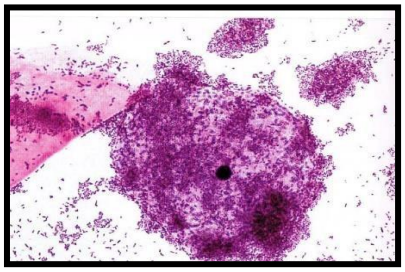
Case 3. Assess the vaginal microbiota from a smear with a known pH in the following documented microscopic cases (enter: increased number, absent, normal, etc.).



Leukocytes – .....
Lactobacillus spp. – .....
Other bacteria – .....
Yeast cells – .....
Epithelial cells – .....
pH – .....

Interpretation:

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